Estrogenic and Anticonceptive Activity of a Petroleum Ether Soluble Fraction of *Malvaviscus conzattii* Greenum Flowers in Female Rats

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A petroleum ether soluble fraction Fr. III isolated from the benzene extract of *Malvaviscus conzattii* Greenum flowers and administered orally to adult female rats at the doses of 10, 20 and 40 mg/rat from day 1 to day 5 of pregnancy showed 50%, 60% and 80% anti-implantation, as well as 60%, 80% and 100% anticonceptive activity in the treated animals, respectively. There was a dose dependent termination of pregnancy and reduction in the number of implantation sites. In ovariectomized young female rats, the Fr. III exhibited estrogenic activity at comparable anticonceptive doses, but was devoid of antiestrogenic activity. Fr. III seems to possess a weak estrogenic activity in that a significant uterotrophic effect was discerned even at 1/16th of the anticonceptive dose.

Keywords: **Anti-implantation; anticonceptive; *Malvaviscus conzattii*; female ovariectomized rats; estradiol-17β.**

INTRODUCTION

Research interest in medicinal plants and the products derived from them has increased substantially over the past few years and the need for an effective and safe contraceptive from plants is very well recognized (Bhargava, 1986a).

Investigations into the effects of prolonged treatment with *Malvaviscus conzattii* Greenum flower extract in the bat (*Rhinopoma kinneari* Wroughton) and dog (*Canis familiaris* Linn.) have led to the observation that the flower extract produces a selective effect on the male reproductive system (Dixit and Bhargava, 1978; Dixit *et al.*, 1978). Although data from other laboratories have confirmed the female antifertility activity of *Hibiscus rosa sinensis* Linn. (family Malvaceae) (Kholkute and Udupa, 1976; Kholkute *et al.*, 1976), the same has yet to be confirmed for *M. conzattii* Greenum, also a member of the family Malvaceae.

The present paper reports the postcoital anticonceptive efficacy in female rats of the petroleum ether soluble fraction Fr. III of a benzene extract of *M. conzattii* flowers.

MATERIALS AND METHODS

Extraction and fractionation

Malvaviscus conzatti Greenum flowers collected from the Ramniwas garden, Jaipur, during the winter season were air dried, extracted 3 times with 90% EtOH by cold percolation. The combined extracts were concentrated to an oil. The oil was then systematically fractionated into C_6H_6-soluble Fr. I and C_6H_6-insoluble Fr. II. The Fr. I was further fractionated with petroleum ether to yield the petroleum ether soluble Fr. III and the petroleum ether insoluble Fr. IV.

Experimental animals

Both adults and young female Wistar rats weighing 175–225 g and 90–110 g, respectively, were employed in this study. Animals were housed in a well-ventilated room (temperature 23 ± 2 °C, humidity 65–70% and 12 h dark/light) and fed with standard commercial rat chow [Hind Lever, Bombay, India] and tap water *ad libitum.*

Postcoital anticonceptive test

Adult female rats having a regular estrous cycle were taken and mated overnight with proven fertile males using a 3:1 (F/M) ratio in the proestrous phase of the cycle. The estrous cycle was monitored by daily vaginal smears taken between 6–8 a.m. Only animals exhibiting at least three consecutive 4 day estrous cycles were used in this test, as described previously (Bhargava *et al.*, 1984). Next morning, the presence of clumps of spermatozoa in the vaginal smear confirmed the mating and was termed as day 1 of pregnancy (D_1). On day 1 to day 5, different doses of different fractions were administered by gavage as shown in Table 1. A control group of mated female rats received an equal volume of vehicle (each test material was macerated with an equal amount of 1% gum acacia and suspended in distilled water) for the same period.

Laparotomy of all rats was done on day 10 of pregnancy (D_{10}) under light ether anesthesia and the number of implantation sites in both horns of the uterus was recorded. The wound was sutured and the rats were allowed to go to term. The number of pups delivered by the dams naturally at the completion of term was noted. The rats were reopened on day 22 (D_{22}) to study any possible delayed effects of different fractions after D_{10}.

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Estrogenic and antiestrogenic tests

In earlier studies it has been shown that the postcoital effectiveness of certain agents will roughly parallel their estrogenicity (Jacob and Morris, 1969; Bhargava, 1984, 1986b). Therefore, estrogenic and antiestrogenic tests were performed for Fr. III using the methods of Rubin *et al.* (1951) and Edgren (1975), respectively. In both tests, 15 day bilaterally ovariectomized young rats were used and estradiol-17β was taken as a reference hormone.

In the estrogenic studies, different doses of estradiol-17β were prepared 30 min before intramuscular injection using olive oil as the vehicle. The different doses of Fr. III were prepared by suspending in 1% gum acacia and were administered by gavage to rats.

To determine antiestrogenic activity of Fr. III, its different doses were administered orally to rats receiving at the same time a uniform dose (1.0 μg/rat) of estradiol-17β intramuscularly.

Separate controls received the vehicle(s) only, i.e. either olive oil or 1% gum acacia or both.

Data (Mean ± SEM) for the relative uterine weights of the different experimental groups were compared with the control groups using the Student's t-test. A p value of 0.05 or less was taken as the criterion of significance.

RESULTS AND DISCUSSION

The results obtained with different fractions of benzene extract of *M. conzattii* flowers (number of implantation sites and number of pups delivered per rat) are shown in Table 1.

Oral administration of 90% EtOH extract of *M. conzattii* flowers to mated female rats at a daily dose of 40 mg/rat on days 1–5 postcoitum caused 50% anti-implantation and 60% anticonceptive activity. On further fractionation the anticonceptive activity was localized in the petroleum ether soluble fraction Fr. III. It was observed that at the doses of 10, 20 and 40 mg/rat on days 1–5 postcoitum the administration of the petroleum ether soluble fraction Fr. III resulted in 50%, 60% and 80% anti-implantation, as well as 60%, 80% and 100% anticonceptive activity, respectively (Table 2).

All the mated animals delivered normally, and none of the pups delivered showed any evidence of teratogenicity up to the age of 1 month.

When the petroleum ether soluble fraction Fr. III was administered orally to bilaterally ovariectomized young female rats for five consecutive days, it induced a dose dependent increase in uterine weight, premature opening of the vagina and cornification of the vaginal epithelium. At the 20 mg/rat dose (equivalent to the 40 mg/rat anticonceptive dose in adult rats), the Fr. III induced a marked stimulation in the uterine weight relative to the controls ($p < 0.001$, Table 3). At this same dose, most of the animals (80%) had an open vagina and the vaginal smear consisted of cornified and rounded epithelial cells. A significant uterotrophic effect ($p < 0.001$) was evident even at 1/16th (1.25 mg) of the comparable anticonceptive dose of Fr. III (20 mg). Premature opening of the vagina and appearance of cornified cells in the vaginal smears were first discerned at the 5 mg dose. Taking a double increase in uterine weight as a measure of

Table 1. Effect of *M. conzattii* extract and its different fractions on number of implantation sites and pups delivered in adult female rats

Treatment[a] (Fraction)	Dose (mg/rat)	Quantal pregnancy	No. of implantation sites per rat[b,c]	No. of pups delivered per rat[d]
Control	—	10/10	8, 8, 6, 8, 7	7, 8, 5, 8, 6
			9, 8, 6, 7, 6	7, 8, 6, 7, 6
M. conzattii (90% EtOH)	40	5/10	0, 7, 6, 0, 0	0, 7, 5, 0, 0
			0, 5, 4, 0, 4	0, 5, 0, 0, 3
Benzene soluble (Fr. I)	20	5/10	5, 0, 0, 0, 0	5, 0, 0, 0, 0
			6, 0, 3, 2, 2	5, 0, 0, 2, 0
	40	4/10	0, 4, 2, 0, 0	0, 4, 0, 0, 0
			4, 0, 0, 2, 0	4, 0, 0, 0, 0
Benzene insoluble (Fr. II)	20	9/10	7, 5, 5, 6, 6	6, 5, 5, 5, 5
			0, 6, 6, 5, 5	0, 6, 5, 5, 5
	40	8/10	5, 5, 4, 6, 4	5, 5, 3, 4, 0
			5, 7, 0, 0, 5	5, 7, 0, 0, 4
Petroleum ether soluble (Fr. III)	10	5/10	0, 4, 0, 0, 2	0, 4, 0, 0, 0
			0, 4, 3, 2, 0	0, 4, 2, 2, 0
	20	4/10	3, 0, 3, 0, 0	2, 0, 0, 0, 0
			0, 0, 0, 2, 2	0, 0, 0, 2, 0
	40	2/10	2, 0, 0, 0, 0	0, 0, 0, 0, 0
			0, 0, 3, 0, 0	0, 0, 0, 0, 0
Petroleum ether insoluble (Fr. IV)	20	8/10	7, 8, 6, 8, 7	6, 6, 6, 8, 0
			7, 6, 0, 0, 7	7, 6, 0, 0, 7
	40	7/10	7, 0, 5, 0, 6	6, 0, 0, 0, 5
			6, 0, 5, 5, 6	5, 0, 5, 4, 5

[a] Days 1–5 of pregnancy, oral

[b] Day 10 of pregnancy

[c] Zero values are data taken for calculating percentage of anti-implantation activity (see Table 2).

[d] Zero values are data taken for calculating percentage of anticonceptive activity (see Table 2).

Table 2. Anti-implantation and anticonceptive activity of *M. conzattii* extract and its different fractions in adult female rats

Treatment[a] (Fraction)	Dose (mg/rat)	Implantation sites per rat (mean ± SEM)[b]	Pups delivered per rat (mean ± SEM)	Animals with anti-implantation activity (%)	Animals with anticonceptive activity (%)
Control	—	7.3 ± 0.11	6.8 ± 0.10	0	0
M. conzattii					
(90% EtOH)	40	2.6 ± 0.13	2.0 ± 0.27	50.0	60.0
Benzene soluble	20	1.8 ± 0.23	1.2 ± 0.21	50.0	70.0
(Fr. I)	40	1.2 ± 0.17	0.8 ± 0.17	60.0	80.0
Benzene insoluble	20	5.0 ± 0.19	4.7 ± 0.17	10.0	10.0
(Fr. II)	40	4.1 ± 0.23	3.3 ± 0.25	20.0	30.0
Petroleum ether soluble	10	1.5 ± 0.17	1.2 ± 0.17	50.0	60.0
(Fr. III)	20	1.0 ± 0.13	0.4 ± 0.08	60.0	80.0
	40	0.5 ± 0.11	NIL	80.0	100.0
Petroleum ether insoluble	20	5.6 ± 0.30	4.6 ± 0.32	20.0	30.0
(Fr. IV)	40	4.0 ± 0.28	3.0 ± 0.26	30.0	40.0

[a] Days 1–5 of pregnancy; oral
[b] Day 10 of pregnancy

estrogenicity, Fr. III orally was found to have about 0.6×10^{-4} times the estrogenic potency of intramuscular estradiol-17β. In the case of estradiol-17β, a double increase in uterine weight was observed at 0.09 μg dose (from 58.8 to 117.6 mg/100 g body wt). With Fr. III this increase was seen at 1.41 mg dose (from 57.6 to 115.2 mg/100 g body wt) (Table 3).

As shown in Table 4, the animals of the control group had a relative uterine weight of 58.6 mg with closed vagina and an absence of any cornified cells in the vaginal smear. Intramuscular estradiol-17β (1.0 μg/rat per day) induced a highly significant increase in uterine weight in the ovariectomized rats. There was a premature opening of the vagina in all these animals and most of the cells in their vaginal smears were cornified. The effects after administration of the same dose of estradiol-17β in combination with different doses of the Fr. III were essentially of the same order as that seen with estradiol-17β alone.

The petroleum ether soluble fraction Fr. III isolated from the benzene soluble fraction from the flowers of *M. conzattii* possesses postcoital anti-implantation and anticonceptive activity in pregnant rats when administered orally during the implantation period. In young ovariectomized female rats, Fr. III exhibited definite estrogenic activity at the comparable anticonceptive dose and was devoid of any antiestrogenic capacity. This frank estrogenicity of petroleum ether soluble fraction Fr. III of *M.*

Table 3. Determination of estrogeneic activity of petroleum ether soluble fraction (Fr. III) of *M. conzattii* in young ovariectomized female rats

Treatment and dose/rat	Relative uterine weight (mg/100 g body wt)	Quantal open vagina	Vaginal smear analysis[d]		
			Leucocytes	Epithelial cells	Cornified cells
Controls I	57.6 ± 2.4	0/10	++++	–	–
Controls II	58.8 ± 2.9	0/10	++++	–	–
Estradiol-17β					
0.10 μg	130.3 ± 6.3*	0/5	++++	–	–
0.25 μg	161.6 ± 9.7[a]	2/5	++	+	+
0.50 μg	220.4 ± 15.6[b]	3/5	+	++	+
1.00 μg	323.3 ± 17.1[c]	5/5	–	+	+++
5.00 μg	371.7 ± 21.6[a]	5/5	–	–	++++
Malvaviscus conzattii (Fr. III)					
0.40 mg	71.2 ± 2.9	0/5	++++	–	–
1.25 mg	102.0 ± 4.7*	0/5	++++	–	–
2.50 mg	135.1 ± 7.5[b]	0/5	++++	–	–
5.00 mg	192.8 ± 12.4[c]	2/5	++	+	+
10.00 mg	261.3 ± 14.1[c]	3/5	+	++	+
20.00 mg	290.5 ± 14.5	4/5	–	+	+++
40.00 mg	308.9 ± 16.6	4/5	–	+	+++

Values are mean ± SEM Controls I (1% gum acacia orally) served for comparison with Fr. III groups and controls II (Olive oil intramuscularly) served for comparison with estradiol-17β groups. Treatment was daily for 5 days (Fr. III = oral; estradiol-17β = intramuscular).
Statistical significant: vs. Controls, $^{*}p \leq 0.001$; vs. preceding value
[a] $p \leq 0.05$
[b] $p \leq 0.01$
[c] $p \leq 0.01$
[d] Rating scheme for vaginal smears: – absent; + < 25%; ++ 25–50%; +++ 50–90%; ++++ 100%.

Table 4. Determination of antiestrogenic activity of petroleum ether soluble fraction (Fr. III) of *M. conzattii* in young ovariectomized female rats

Treatment and dose/rat		Relative uterine weight (mg/100 g body wt)	Quantal open vagina	Vaginal smear analysis[a]		
				Leucocytes	Epithelial cells	Cornified cells
Control		58.6 ± 2.3	0/10	++++	–	–
Estradiol-17β (1.0 μg/rat)		323.3 ± 17.1	5/5	–	+	+++
Malvaviscus conzattii + Fr. III (mg/rat)	Estradiol–17β (μg/rat)					
0.50 +	1.0	331.5 ± 18.4	5/5	–	–	++++
1.25 +	1.0	354.3 ± 20.7	5/5	–	–	++++
2.50 +	1.0	360.1 ± 19.5	5/5	–	–	++++
5.00 +	1.0	343.2 ± 15.9	5/5	–	–	++++
10.00 +	1.0	321.7 ± 11.4	5/5	–	–	++++
20.00 +	1.0	315.5 ± 11.1	5/5	–	–	++++
40.00 +	1.0	307.4 ± 10.3	5/5	–	–	++++

Values are mean ± SEM. Controls (receiving 1% gum acacia orally + olive oil intramuscularly) served for comparison with *M. conzattii* (Fr. III) + estradiol-17β group. Treatment was daily for 5 days (Fr. III = oral; estradiol-17β = intramuscular).

Statistical significance: vs. controls, $^{*}p \leq 0.01$; vs. preceding value, comparisons not significant; vs. 1.0 μg estradiol-17β injected group, comparisons no significant.

[a] Rating scheme for vaginal smears: – absent; + <25%; ++ 25–50%; +++ 50–90%; ++++ 100%

conzattii may account for its anticonceptive activity in rats. Subsequent to these present studies involving the estrogenic properties of the petroleum ether soluble fraction Fr. III of *M. conzattii* a comparison of the anticonceptive ability in females with antispermatogenic activity in males would be a useful extension of the work. As reported earlier (Dixit and Bhargava, 1978; Dixit *et al.*, 1978) the oral administration of the alcoholic extract of *M. conzattii* flowers (50 mg/animal/day for 2 weeks in bats and 150 mg/kg/day for 8 weeks in dogs) induced selective degeneration of the latter stages of spermatogenesis, for example, the spermatocytes, spermatids and spematozoa were vulnerable to the extract treatment. The spermatogonia and sertoli cells were free from degeneration.

From this investigation, carried out on female rats, together with earlier published work on male dogs and bats (as discussed above) it seems that *M. conzattii* flowers are potentially contraceptive in action when used in both males and females.

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